

Claims

1. An isolated nucleotide sequence responsible for the tomato *high pigment 1* (*hp-1*) phenotype, wherein said sequence comprises an altered tomato *DDB1* gene sequence or fragment thereof, wherein said the alteration in said altered sequence or fragment comprises an A-to-T transversion at nucleotide 931 of said *DDB1* gene sequence.

2. The isolated nucleotide sequence according to claim 1, wherein said sequence comprises the sequence defined as SEQ ID NO:1 in the sequence listing.

3. The isolated nucleotide sequence according to claim 1, wherein said sequence comprises a fragment of SEQ ID NO:1, and wherein said fragment comprises nucleotide 931 of the *DDB1* gene sequence.

4. An isolated nucleotide sequence responsible for the tomato *high pigment 1<sup>w</sup>* (*hp-1<sup>w</sup>*) phenotype, wherein said sequence comprises an altered tomato *DDB1* gene sequence or fragment thereof, wherein said the alteration in said altered sequence or fragment comprises a G-to-A transition at nucleotide 2392 of said *DDB1* gene sequence.

5. The isolated nucleotide sequence according to claim 4, wherein said sequence comprises the sequence defined as SEQ ID NO:2 in the sequence listing.

6. The isolated nucleotide sequence according to claim 4, wherein said sequence comprises a fragment of SEQ ID NO:2, and wherein said fragment comprises nucleotide 2392 of the *DDB1* gene sequence.

7. A method for detecting the presence of the *hp-1* mutation in a plant, comprising the steps of isolating the genomic DNA from said plant, amplifying a gene fragment containing said *hp-1* mutation from said genomic DNA by use of a PCR technique and determining the presence of said *hp-1* mutation in said genomic DNA.

8. The method according to claim 7, wherein the presence of the *hp-1* mutation is determined by the use of a pyrosequencing technique, and wherein the sequence data obtained from said technique is compared with the sequence defined in SEQ ID NO:1.

9. The method according to claim 7, wherein the plant in which the presence of the *hp-1* mutant is being detected is of the species *Lycopersicon esculentum*.

10. A method for detecting the presence of the *hp-1<sup>w</sup>* mutation in a plant, comprising the steps of isolating the genomic DNA from said plant, amplifying a gene fragment containing said *hp-1<sup>w</sup>* mutation from said genomic DNA by use of a PCR technique and determining the presence of said *hp-1<sup>w</sup>* mutation in said genomic DNA.

11. The method according to claim 10, wherein the presence of the *hp-1<sup>w</sup>* mutation is determined by the use of a pyrosequencing technique, and wherein the sequence data obtained from said technique is compared with the sequence defined in SEQ ID NO:2.

12. The method according to claim 10, wherein the plant in which the presence of the *hp-1<sup>w</sup>* mutant is being detected is of the species *Lycopersicon esculentum*.

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13. Use of the method according to either claim 7 or claim 10 as a means of post-control in seed production.

14. A method for the determination of the presence of two different photomorphogenic mutations in a plant, wherein one of said mutations is either the *hp-1* or the *hp-1<sup>w</sup>* mutation, comprising detecting the presence of a photomorphogenic mutation other than the *hp-1* or the *hp-1<sup>w</sup>* mutation by either genotypic or phenotypic selection means, and detecting the presence of the *hp-1* or the *hp-1<sup>w</sup>* mutation by means of the method according to either claim 7 or claim 10.

15. The method according to claim 14, wherein the phenotypic selection means for determining the presence of the non-*hp-1*, non-*hp-1<sup>w</sup>* photomorphogenic mutation comprises germinating seeds obtained from the plant in which the presence of the mutations is being determined in a temperature controlled chamber, under a yellow plastic screen that is opaque to light having a wavelength less than 500nm, and selecting non-etiolated seedlings.

16. A method for preparing double-mutant lines of *Lycopersicon esculentum* having genotype *hp-1/hp-1 p/p*, wherein *p* represents any recessive photomorphogenic lycopene-enhancing mutation that is genetically unlinked to the *hp-1* mutation, said method comprising the steps of:

- a) cross-hybridization of a homozygous *hp-1/hp-1* line or plant with a homozygous *p/p* line or plant to yield double heterozygous *hp-1/+ p/+* F<sub>1</sub> plants;
- b) self-crossing of the F<sub>1</sub> plants obtained in step (a) in order to yield F<sub>2</sub> seeds;

- c) identification of double homozygous plants *hp-1/hp-1 p/p* by means of the application of the method defined in claim 7 and a method for detecting the presence of the *p* mutation;
- d) self-crossing of the double homozygous plants identified in step (c) to generate  $F_3$  seeds, and germination of said seeds.

17. The method according to claim 16, wherein mutation *p* is the *dg* mutation.

18. The method according to claim 17, wherein the determination of the presence of the *dg* mutation in step (c) of the method is performed using the marker for the *dg* mutation disclosed in co-owned, co-pending application PCT/IL03/00023.

19. A method for preparing double-mutant lines of *Lycopersicon esculentum* having genotype *hp-1<sup>w</sup>/hp-1<sup>w</sup> p/p*, wherein *p* represents any recessive photomorphogenic lycopene-enhancing mutation that is genetically unlinked to the *hp-1<sup>w</sup>* mutation, said method comprising the steps of:

- a) cross-hybridization of a homozygous *hp-1<sup>w</sup>/hp-1<sup>w</sup>* line or plant with a homozygous *p/p* line or plant to yield double heterozygous *hp-1<sup>w</sup>/+ p/+*  $F_1$  plants;
- b) self-crossing of the  $F_1$  plants obtained in step (a) in order to yield  $F_2$  seeds;
- c) identification of double homozygous plants *hp-1<sup>w</sup>/hp-1<sup>w</sup> p/p* by means of the application of the method defined in claim 10 and a method for detecting the presence of the *p* mutation;
- d) self-crossing of the double homozygous plants identified in step (c) to generate  $F_3$  seeds, and germination of said seeds.

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20. The method according to claim 19, wherein mutation p is the dg mutation.

21. The method according to claim 20, wherein the determination of the presence of the dg mutation in step (c) of the method is performed using the marker for the dg mutation disclosed in co-owned, co-pending application PCT/IL03/00023.

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